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Robert Hahl

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: James J. Hickman

Examiner: M. P. Allen

Serial No.: 09/575,377

Art Unit. 1631

Filed: May 22, 2000

For: HIGH THROUGHPUT FUNCTIONAL GENOMICS

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
Washington, DC 20231
Sir:

I, Prof. James J. Hickman, Ph.D., do hereby make the following declaration:

1. I am the same James J. Hickman, Ph.D., who previously submitted a Declaration under 37 C.F.R. §1.132 in the above-captioned application.

2. I am currently Director of Nanoscience Technology, and Professor of Chemistry and Biomolecular Science at The University of Central Florida.

3. Impedance is a quantitative measurement of the electrical characteristics at, in the context of this invention, the interface between a cell membrane and an electrode. It is composed of a resistance term and a capacitance term and is proportional to $1/R_e$ where R_e is the *electrode* resistance. Impedance is maximized when R_e is minimized. However, Impedance is also affected by the

resistance of the seal, R_{seal}, as indicated in Figure 8 of this application. When R_{seal} is small, the current from the surrounding bath is high and the impedance signal at the microelectrode is reduced, which is undesirable. When R_{seal} is high, it serves to maximize the Impedance at the microelectrode. These relationships are explained in detail on page 47 of the application.

4. By referring to a "high impedance seal" in the context of this invention, one is also describing a high resistance seal, by definition, because the impedance is maximized when the seal resistance is maximized. Thus, the terms "high impedance seal" and "high resistance seal" are synonymous in this invention.

5. Patch-clamp electrophysiology is a standard technique for measuring the electrical properties of an excitable cell including the electrical components in the cell membrane and the primary ion channels. This technique was developed by Neher and Sakmann and is described in detail in an article they wrote after receiving the Nobel Prize in 1991 ("The Patch Clamp Technique," Scientific American, 1992, 266.44-51.) One of the necessary steps to enable patch-clamp electrophysiology is the creation of a high resistance or high impedance seal between a glass micropipette and the cell membrane. This kind of seal is achieved in the patch-clamp technique by pulling a slight vacuum in the micropipette, but it can be achieved by other means as well.

6. Provisional Application 60/135,275 states: "Specific Aim 2 involves localizing the neuronal cells on the microelectrodes of a microelectrode array with surface chemistry to establish a patch-clamp like seal between the cells and the microelectrode" (page 28, lines 22 - 24) Here the phrase "patch-clamp like seal" describes a high resistance or high impedance seal to a person of skill in the art.

7. Deconvolution is a mathematical tool for separating an electronic or similar signal into its component parts using fitting routines to maximize agreement between the original signal and to estimate contributions from the component parts. In the present invention, separate components of the action potential arise from the contributions of several different ion channels and how they relate to the pathways inside a cell, as depicted in Figure 4 of the specification. Thus, deconvolution

analysis reveals aspects of the basic cell biology. Implementation of this technique is depicted schematically in Fig. 10, in the box labeled "Model output AP S38."

8. Deconvolution is a very different process from Fourier-transform analysis, which seeks the best fit for a series of sine waves to the signal to create a signature. Such sinusoidal wave forms have no relevance to the biological components giving rise to an action potential, and the signature generated by a Fourier-transform analysis could not be readily related to changes in the ion channel flux.

9 To deconvolute an action potential into separate contributions from the three or more primary ion channels of interest in the present invention requires a high impedance seal because the greater the impedance, the greater the signal strength and the lower the noise. A high signal-to-noise rano gives one confidence when deconvoluting the contributions of each ion channel and when determining how each channel changes in response to variations of the cell's environment. As with all electronic measurements of this type a weak signal or a large noise component decreases the confidence one has in the analysis of the signal. Thus, the required impedance value for a device of the invention is one high enough to allow reliable discrimination of changes in the signal in a deconvolution analysis.

10. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 11-26-04

By: _____
Prof. James J. Hickman